04/22/2024

Summary 1th Meeting on Empty Nose Syndrome

INTRODUCTION:

The meeting was to be recorded for team use and possible sharing with other professionals if required. Therefore, all participants had to give their consent.

Presentation of the multidisciplinary team working on the ENS project

- Prof. Pellegrini's research group, Centre for Regenerative Medicine, University of Modena and Reggio Emilia, Italy: with extensive clinical experience in the regeneration of many epithelial tissues (cornea, skin, urethra; oral mucosa...). Prof. Pellegrini, leader of the cell therapy group, is also working on airway regeneration.
- The research group of Prof. Martin/Barbero, University Hospital Basel, Switzerland: With strong translational experience in cartilage regeneration, from basic science to clinical application.
- Clinicians from Switzerland and Italy, specialised in otolaryngology.
- Stefania Bettinelli: Founder of "Le ail di Camilla", a non-profit patient support organisation (based in Modena), not linked to any specific pathology, with the aim of supporting research and patients in terms of practical issues, but also externalisation and communication.
- Other specialists: from different multidisciplinary sectors, helpful in achieving the project's objectives.

OBJECTIVES OF THE MEETING

- Getting to know each other, introducing our team and building a network. No results are possible without networking and collaboration. Therefore, close cooperation is needed between all stakeholders: patients, scientists, clinicians and all other professionals.
- Describe our approach and results, the challenges involved and the steps that still need to be taken.
- Understand the issues that need to be addressed and the expectations/priorities of patients. Give us your view of the pathology and what is relevant.
- Learn from previous experience (previous reconstructive strategies, complications, surgeons involved and types of surgery). It is very important for us to avoid any kind of underestimation or repetition of a wrong step.
- No results can be achieved without the close cooperation of all stakeholders (patients, scientists, clinicians and any other specialists required).
- Not all questions will be answered during the first meeting, we propose regular updates and exchanges of information with specific timetables (approximately every six months, as science needs time to produce certainties).

THE RATIONALE OF OUR APPROACH

The rationale for our approach is based on regenerative medicine, the ability to use the regenerative properties of our bodies to rebuild in vitro what is missing. We will try to restore the state that existed before the damage/pathology. So the idea is to do regenerative medicine: to rebuild in vitro what is missing in the body, trying to restore as much as possible the original state or, if necessary, gene therapy, where we go to modify the genome to find the solution to pathologies.

Starting Material:

Our aim is to rebuild the missing part using your own self-renewing cells. **Important: autologous cells, which means we do not use any foreign material that could affect your well-being**. We start with the cells of the specific patient we want to treat. We stimulate the cells in vitro to express their normal regenerative and differentiation potential. So, the capacity that you have inside your cells in your body with the specific molecules defined by your genome, nothing that can alter your normal physiology. Of course, the difficult part is to recreate the microenvironment outside the human body. Once the tissue or organ has been regenerated, the next step is to transplant it in vivo, into the patient's body. However, because the regenerated tissue is made from the patient's own (autologous) cells, the transplant is not subject to the risk of rejection or immune reaction. On the contrary, once transplanted and engrafted, the regenerated tissue will progressively restore the missing function, even through cell-to-cell signalling. The next step will be to follow the patient and monitor the mechanism of action and any reactions the patient may have.

Differences in our approach to augmentation procedures:

It is important to stress that this is not an augmentation procedure. This is something completely different. Augmentation aims to reduce the amount of free space in the nasal cavity. Our approach is to recreate a microenvironment that resembles its natural counterpart. This means restoring the biosystem (not just augmenting it) by inserting autologous living cells from the reconstructed tissue that can communicate with each other and restore the environment. It's a completely different approach, but it's also much more difficult because everything has to be under control and functioning as in normal physiology.

Specific stem cell definition and classification:

The cells with a higher regenerative potential, i.e. the ability to rebuild tissue, are the body's own stem cells.

At present, three types of stem cells are used in therapy:

1) **Embryonic stem cells**: found in the first stages of embryonic development. We will NOT be using this type of cell as they are not autologous.
2) **IPS cells (induced pluripotent stem cells):** Cells that are not naturally present during embryonic development or in the adult human body. These cells are created in the laboratory by genetically modifying the patient's cells to make them similar to embryonic stem cells. However, in the case of Empty Nose Syndrome, it is not necessary to use these transformed cells as we can use the following type of stem cells.

3) **Adult autologous stem cells**: specific cells found in the different tissues of the adult human body that are responsible for the physiological regeneration of their specific tissue. Our team has extensive experience in regenerating many tissues using the enormous power that these cells possess. These cells are safe because there is no risk of genetic modification and these cells already know what their job is, which is to regenerate a specific tissue. In fact, if we do not interfere with their function, they spontaneously regenerate their tissue of origin because they retain the memory of the original site from which they came.

Focus on ENS project:

The idea is to try to rebuild the missing part of the respiratory system, which is quite complex. In particular, the idea is to rebuild the nasal turbinate as closely as possible to its normal counterpart, which is made up of respiratory epithelium, nerves and blood vessels, and bone. Our approach will start with a simplified version of the tissue, which will be progressively increased as a ladder of complexity.

The airway epithelium is a very complex and coordinated system, with many different cell types performing their specific roles very well under physiological conditions. Overall, the main cell types that make up the airway epithelium are derived from the same cell type, known as basal cells because of their basal location in the tissue. This means that if we take some basal cells, we can help them to do their job in vitro and produce many other different cell types. The approach we are taking is to harness the enormous potency of cells, particularly stem cells (a subset of basal cells). The regeneration process starts with a small biopsy, which is the removal of a small piece of tissue from the same patient under anaesthetic to collect some autologous basal stem cells. The tissue is then transported to the laboratory in a special transport medium, where it is dissociated to extract individual cells, which are then fed with specific nutrients to make them multiply and expand to reach the number of cells needed to reconstruct the entire tissue.

Previews successful experiences:

In the past, we have demonstrated the ability to regenerate several different tissues.

In particular, Prof. Pellegrini's group has more than thirty years of experience in the regeneration of human epidermis: transplanted in hundreds of burn patients (De Luca et al. 2006, cell therapy), for the treatment of the devastating genetic disorder epidermolysis bullosa after genetic correction (Mavilio et al. 2006; Hirsch et al. 2017, gene therapy), as well as the regeneration of corneal epithelium (Pellegrini et al. 2017). al 2006; Hirsch et. al 2017, gene therapy) and corneal epithelium regeneration (Pellegrini et. al 1997; Rama et. al 2010), which is the most advanced product as it has been registered and approved at European level under the name Holoclar without any concerns from health authorities (Pellegrini et. al 2018).

Prof Martin/Barbero's group has extensive experience in cartilage regeneration and the transplantation of cartilage tissue to various injured areas of the body, such as the nose or the knee (Fulco et al., 2014; Acevedo Rua et al., 2021; Mumme et al., 2016).

Everything we do is done after very rigorous evaluation, proven and extensively reviewed by health professionals and the international scientific community and published in scientific publications, we

do not charge for treatment: all treatment is done in hospitals. The main focus is to avoid any kind of adverse event or patient suffering.

Cultivation and expansion of human airway epithelial cells

The project to regenerate respiratory epithelium began many years ago with work on the trachea and bronchi. Subsequently, after contact with some patients suffering from ENS, we also started to study the epithelium of the turbinates. We thought that by combining the previous experience of the groups of Prof. Pellegrini and Prof. Martin/Barbero, and thanks to the support of the patients, we could develop an approach that could potentially address this important/urgent medical need.

Extraction of airway epithelial cells:

The starting material is a small nasal airway biopsy, which is subjected to cell isolation for stem cell extraction, these cells are put into culture for cell expansion, then a part of the cells can be cryopreserved for future experiments (in basic research) or for intervention planning (in a clinical setting), as we have done in other clinical applications.

The results of these years of work have shown that it is possible, starting from a small airway biopsy, to extract the specific autologous adult stem cells of the tissue, to maintain this powerful population in vitro and to obtain an extremely good and effective cell expansion in vitro.

In vitro airway epithelium regeneration:

The expanded cells were used to regenerate airway epithelium, attempting to recapitulate the complexity of this tissue. We were pleased to find that the regenerated epithelium contained all the main cell types that characterise the native counterpart. Namely, basal cells at the bottom of the tissue and ciliated cells at the top, able to move the mucus produced by functional goblet cells. Most importantly, the regenerated epithelium also contained stem/progenitor cells capable of self-renewing the in vitro cultured tissue.

Regenerated epithelium functionality:

The regenerated airway epithelium is physiological and functional. In fact, when we simulated an in vitro wound in the epithelium, the cells were able to coordinate with each other and close the wound in a very short time. More importantly, the airway epithelial cells were able to form a barrier capable of protecting the inner part of the body from any type of contaminant from the inhaled air.

Regeneration of cartilage tissue:

Similarly, the cartilage regeneration process begins by taking a very small piece of nasal septum cartilage biopsy from the patient who needs cartilage reconstruction (autologous treatment). The matrix is then removed to release the cells from the biopsy. The extracted cells are cultured in vitro to increase their number, producing more than 100 million cells in two weeks. These cells are then used to create the engineered cartilage by colonising a natural collagen sponge (already clinically approved). During this engineering phase, the cells are induced to produce their own matrix, allowing a tissue similar to native cartilage to be produced in the laboratory. In just four weeks, these

engineered cartilage grafts contain not only living chondrocytes, the specific cells of cartilage, but also the newly produced extracellular matrix component, which is essential for tissue architecture and function. As with airway epithelium, it took a long time to generate and develop the protocol to produce the cartilage. We started by evaluating the best cell source to generate this piece of cartilage, looking at chondrocytes from different parts of the body. These studies showed that nasal chondrocytes (which are not stem cells but tissue-specific cells) have a very high and reproducible proliferative and cartilage-forming capacity (Tay et al, 2004; Candrian et al, 2008). These cells have a good capacity to survive, integrate and function when transplanted into the tissue environment (Pelttari et al., 2014), most likely due to their neural crest-derived origin. These cells also have a high capacity to resist inflammatory stress (Scotti et al., 2012; Acevedo Rua et al., 2021). Thanks to all these properties, our engineered cartilage grafts generated with nasal chondrocytes have already been used in two clinical trials conducted at the University Hospital of Basel for the treatment of various nasal cartilage problems [Phase I clinical trial, Nose-2-Nose (TpP-I-2010-002) Fulco & Miot et al., Lancet (2014); Phase I clinical trial; NSP (NCT04633928) Kaiser et al., International Journal of Surgery (under revision)].

Overview of the whole turbinate reconstruction approach:

The procedure under development involves taking a small biopsy of cartilage and a small biopsy of airway epithelium; the amplification procedures will be carried out in the Modena and Basel laboratories, producing the final construct resembling the native turbinate.

This approach has already been carried out on several donor biopsies, producing a construct composed of cartilage tissue with a good structural and biological composition (containing all the specific matrix proteins) and a normally differentiated airway epithelium, with all the different cell types such as ciliated cells, goblet cells and all the other cell types that characterise the native respiratory epithelium in vivo.

In the future, when everything is perfectly defined, the idea is to transplant this construct and restore normal physiology in the nose. One of the great strengths of this project is that all the stimuli we give the cells to help them through the regeneration process are already clinically validated. In fact, the culture conditions used for cartilage regeneration have been approved by the health authorities in Switzerland, while the culture conditions we are using for the epithelium have been approved at the European level. Therefore, we are already using safe and approved conditions for the human cells to be transplanted.

Next steps:

Not everything is finished! There is still a lot of work to be done! The next steps that will guide us in the coming period are

- **Testing compatibility** with the blood vessels and nerves that are essential for the construct to function in the body.
- **Produce** the appropriate size and shape of the construct to be defined with clinicians. The models will be used to study different surgical approaches. We have no size limit as cell culture expansion is extremely efficient, but the size will be determined by the type of surgery and the body's response.

- **Define** the appropriate surgical protocol to maximise patient recovery and return of nasal function. This step requires extensive consultation with several experts.
- **Consultation** with regulatory authorities to define preclinical and clinical studies to monitor the safety and efficacy of the construct. Nothing will be done in a private clinic, this would be done in a standard hospital and will be approved by the health authorities to avoid any kind of problem to you and any kind of lack of efficacy.
- **Pre-clinical study**: usually agreed with the regulatory authorities.
- **Standardisation** of the manufacturing process needed to move from a pre-clinical to a clinical setting.
- **Clinical trials**.

IMPORTANT: At any point in the process, there might be something to fix or tweak. This process takes a lot of patience. The regular update will help to understand where we are in the process. What is the problem and what is the forecast for the solution. But in the meantime, because research takes a lot of effort, we have short term goals that may be relevant to you.

SHORT-TERM GOALS: **EDUCATION AND AWARENESS**

- Publish reviews of the literature with a special focus on ENS problems and treatments, to update doctors who are not aware of your pathology, to address the problems and to avoid repeating mistakes in case some treatment did not work properly.

- **Scientific manuscripts** on new results: to draw attention to this type of pathology and to the new knowledge that we will be able to provide.

- **Presentations and posters** at conferences to raise awareness of the syndrome and the urgent medical need for therapeutic solutions.

- **Regular updates** on our project will support our collaboration, exchange of opinions/ideas, experiences in order to be aligned.

- Preparation of **questionnaires** and possible re-evaluation of patients' conditions

- Creation of a **database** to optimise procedures, making all known information on diagnosis and treatment available on a single platform.

Fund and resources:

Research has a cost therefore it is relevant to attract funds and resources.

- PRIN (national relevant project of general interest) 2022 (on-going)
- Support of Le Ali di Camilla patients' association for rare diseases
- Application to other national and international grants with the support of patients' association and collaboration of patients.

How to communicate with us and receive updates

Communication is extremely important, but it is also important to give us some rules.
It's not possible to focus on research activities, funding, writing and communication at the same time, so we will communicate at scientific meetings and through the following channels
and within certain time frames through the following channels

- WhatsApp group "Le Ali di Camilla ENS" to communicate about future dates
- Semi-annual meetings for scientific and educational updates, questions, and answers.

QUESTION AND ANSWERS

1. ***Where is the tissue taken from?***

In the experiments we are performing we start with biopsies of donors (patients who, undergoing normal surgical procedures, choose to donate the leftover tissue to the research). In real clinical practice, the biopsy, the small piece of tissue to start from, (starting material) will come from the patient that needs to be treated. Each patient who is eligible to be treated will undergo 2 very small biopsies harvesting: one from specific cartilage and one from the nasal epithelium to develop the tissue with the specific cells for the specific patient, as we did in the past for the eye for the epidermis, for the cartilage. We need autologous cells, so cells coming from the same individual to avoid any kind of immunological rejection.

2. ***Which is the maximum tissue transplant that can be done?***

We have no limitation regarding the size of tissue that we can produce. Indeed, with the small biopsy we can produce the entire turbinate and probably more than one. So, the dimension is not an issue. Indeed, by selecting and checking for the presence of stem cells (in the case of epithelia) or tissue-specific cells (in the case of cartilage tissue) within customized culture conditions the cells can reconstruct big structures. This is not an issue for us.

3. ***How much tissue will be taken? Is it possible to take from other harvesting sites, for example, bronchi and costal cartilage?***

In the case of the nasal defect for cartilage regeneration, we used a starting material, a 6 millimetre diameter cartilage biopsy, and it was enough to generate a very large piece of cartilage. We may want to continue using this strategy because it was safe and feasible. However, we may even consider using different starting materials in patients with a very compromised nasal septum. This procedure will be more invasive because it involves several body parts.

The same applies to epithelial biopsy. Even if the patient has a severely damaged turbinate, it is always possible to take a small biopsy to start the procedure. Especially for the epithelium, we only need a few millimetres to have enough stem cells to work with. Therefore, the donor area for the biopsy is not an issue.

4. ***Can the regenerated cartilage be used for the reconstruction of other resected nasal structures for people who have had other areas of the nose over-resected as well as the turbinate?***

Absolutely! In fact, in our previous clinical trial, we used this approach to treat cartilage problems in other parts of the nose. For example, the first clinical trial was to repair the defect in the alar lobe of the nose, and we successfully treated these cases. With our approach, we can repair different types of cartilage defects in the nose, and not just in the nose - we are even using nasal cartilage to treat articular cartilage defects in the knee.

5. ***How do you plan to manage the vascularisation of the implant?***

The idea is to create an environment that can support the migration of vessels and nerves. The idea is to have something that can be autonomously vascularised and reinnervated. Of course, this work should be combined with that of clinicians, because the type of surgery that is used, the previous knowledge and what is done in regular practice and the extent of damage that each patient has is critical!

However, we have carried out some preliminary experiments. A member of the team has spent some time at Harvard University setting up a simplified experimental model that was able to demonstrate the ability of the collagen sponge (used for cartilage regeneration) to be vascularised through the regeneration of new functional blood vessels that are able to integrate into the recipient's body when transplanted in vivo. This is only a starting point, but a very good one.

6. ***Do you have any idea when we might see a clinical application?***

Making predictions in research is always almost impossible. The experiment can have completely different outcomes that are impossible to predict. In addition, the time taken by the regulatory authorities is extremely variable. Different strategies can be evaluated to speed up the process as much as possible. However, it is best to follow the process through regular updates to know what has been done, what is in progress and what remains to be done.

7. ***Have you ever considered the need for soft tissue within the in vivo turbinate structure?***

The need for regeneration of soft tissue between the epithelium and the cartilage is something we have in mind. However, we are still wondering whether it is necessary to stimulate the regeneration of this tissue in vitro by adding other cell types or matrices, as we have observed in our experience with other regenerative processes (e.g. skin) that spontaneous regeneration of connective tissue can occur after transplantation over time. We will investigate both possibilities in detail.

8. ***How can we, as patients, contribute to the research process, beyond providing our medical history in relation to this condition?***

Your input is extremely important and you can contribute in many ways:

- Share your information by filling in the questionnaire that will be sent to you soon.
- Participating as an official team member in research projects during the application process for funding and resources. Serve as a platform to evaluate results and make recommendations for the project. he support of patient associations is highly valued by the committees that evaluate projects and decide who will receive funding.

9. ***Is it possible to donate to the Ali di Camilla?***

Yes, it is possible to donate to this charity. It is important to specify the purpose of the donation, i.e. ENS research. In this way the money will be used for the ENS project. The bank details for the transfer are :

Banco BPM Modena Ag. 7 IBAN: IT37V0503412908000000007123

SWIFT: BAPPIT21503 You can find additional information at https://lealidicamilla.org/dona-ora/

10. Can the FDA give a classification code?

As far as the classification of pathology is concerned, we will be investigating this area both in the US and in Europe. However, with the ongoing research, we can also provide alternative and additional data for the classification definition of the severity level, which could also affect the previous classification of the pathology.

With regard to the classification code for our reconstructive approach, it is still a little early, as the regulatory authorities need to know the details of the manufacturing process before a classification code can be assigned. Classification is therefore only possible when the therapy is complete and ready for use.

11. ENS is inadequately recognized, especially in the US. The informed consent fails to address this important surgical risk, and insurance coverage excludes any form of reimbursement. For instance, if your therapy becomes available in the future, we won't receive reimbursement for the treatment. Can you assist us in addressing this issue?

We can help you in a number of ways:

- Education, writing reviews and original papers on new findings: The literature is distributed worldwide, so the idea of increasing education by writing reviews and papers together with clinicians will increase awareness worldwide, because scientific research is worldwide, not in a specific local place. It will help because no one can ignore the problem once it is in the literature, even if you want to get reimbursed by insurance.

If it's in a lot of scientific papers, they can't ignore it because it's published and available to everyone.

- Involving bioethicists and lawyers: to make informed consent more complete for the patient, more informative, with all the right information. This is absolutely necessary because informed consent is the basis of human rights. We will also include this issue in the scientific papers to raise awareness on this point.

12. What can we, as patients, do to contribute to the research process, apart from providing the teams with our medical history in relation to these pathologies?

Contacting surgeons in different places can be useful if they want to join the project. But we cannot start planning the clinical protocol immediately. However, it would be very helpful to have information from you, because the dissemination of information, together with the classification of the disease and the writing of papers, will help to increase awareness, which also means having more collaboration to speed up the clinical trial and everything else. The first goals will be:

- To make people aware of your disease, what is the problem and what is not involved in this pathology.
- We need to understand what is causing this condition to stop the wrong surgery. Providing any details/information about your condition by filling in the questionnaire will speed up the process.

13. What do you think about using umbilical stem cells, fat grafting, stem cells from adipose tissue, and embryonic/foetal stem cells with collagen hydrogels? What is your position on these treatments for the time being?

To date, we do not have enough scientific data to support any type of stem cell injection. Therefore, we DO NOT encourage patients to undergo any type of experimental protocol that is not sufficiently supported by strong scientific evidence. In fact, the inappropriate use of stem cells may even worsen the patient's condition (see the international guidelines of the International Society for Stem Cell Research: https://www.aboutstemcells.org/).

. We know that some stem cell-based approaches can have some benefits in terms of reducing inflammation and modifying the environment a little bit, which could induce some regeneration. But if the turbinates are completely or even partially affected, injecting fat cells will not be the best choice of treatment for these patients. There are plenty of stem cells in the body, but you have to put the right one in the right place: they are not miracles. In addition, the benefits of injecting fat cells can be very short-lived because the cells die within a few hours of being injected. Therefore, in some cases, the patient may have some relief of symptoms for a very short period of time, but this is not the right approach for proper regeneration. So far, we do not think that any other stem cell-based approach is very convincing. Unfortunately, some people in the stem cell field want to take advantage of patients who are suffering from terrible conditions. They propose stem cell treatments that are not adequately supported by scientific publications and they sell these treatments at a **high price**. These are outright scams, so the advice is to be extremely cautious, always ask for all the scientific evidence behind the proposed therapy, and consult several specialists before embarking on a stem cell treatment (see international guidelines on stem cell treatments).

14. Are you sure that the reconstruction of the whole turbinate is the solution for all kind of patients? Sometimes some patients with less critical conditions can benefit just from a simple implant of cartilage.

We do not suggest one approach for all. There is never a one-size-fits-all approach to any type of therapy. The classification of the pathology and the analysis of the severity of the symptoms of the problem will lead the doctor to decide which treatment is more appropriate. We are not prescriptive. We have an approach to treating extreme cases. If there is a solution for simple cases in the hands of good doctors, that's fine.

CONSIDERATIONS:

The surgical challenge of implanting material: It is extremely difficult to implant anything (scaffold, cartilage implant) in its entire length, especially in the posterior tails and the posterior part of the turbinates, especially if there is damage as in ENS with cauterisation or radiofrequency or laser. In this condition, the airway mucosa becomes very adherent to the bone. This makes it very difficult to detach the mucosa to implant even a millimetre of anything. This is a key challenge that needs to be addressed. What many surgeons do to get around this problem is to implant more anteriorly

because the mucosa is obviously healthier, but that doesn't help the patient. It can actually make the condition worse because if you restrict the airflow anteriorly and it's open posteriorly, it gets to the back of the nose where it's very open even faster. So, this is one of the major surgical challenges at the moment.

Inadequacy of the name ENS:

The name of the disease is confusing and wrong. Empty nose syndrome it does not help convey the severity and gravity of the condition. Moreover, the syndrome is not really related to emptiness. A different name alongside ENS (so that we don't lose the literature and references that we already have) would be helpful.

This is a common problem in other pathologies. Writing papers and giving presentations is one way of proposing a reclassification of the disease with international guidelines for definition and classification. This is a common problem when the pathology is not well known. That is why awareness raising is fundamental.

Psychological/Psychiatric support in case of ENS

We are aware that ENS is not psychological or psychiatric in nature and we will do our best to clarify this concept and raise awareness of this important concept. However, we do not encourage any patient who is currently receiving psychological or psychiatric treatment not to continue with that treatment. It's very important to have support for chronic illness because it helps you. Sometimes the reason why specialists recommend this type of treatment is not because they think that empty nose syndrome or tropic rhinitis is a psychiatric or psychological condition, it's because they know how difficult it is to live with chronic conditions. It's a very frustrating, very challenging process to go through alone and any kind of support, including psychological or even psychiatric, can alleviate the symptoms of coping with this type of chronic disease. If you are receiving any kind of psychological or psychiatric treatment and it is helping you, please continue, it will not really change your situation regarding empty nose syndrome, but it may help you to cope with the problem in general.

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